DESCRIPTION

NOVEL METHODS FOR DETERMINING THE NEGATIVE CONTROL VALUE FOR MULTI-ANALYTE ASSAYS

Cross-Reference to a Related Application

This application claims the benefit of U.S. provisional application Serial No. 60/457,146, filed March 24, 2003.

Background of Invention

Various analytical procedures and devices are commonly employed in assays to determine the presence and/or concentration of substances of interest or clinical significance that may be present in biological liquids or other materials. Such substances are commonly termed "analytes" and can include antibodies, antigens, drugs, hormones, etc.

One frequently used assay format is the immunoassay. Immunoassay techniques take advantage of the mechanisms of the immune systems of higher organisms, wherein antibodies are produced in response to the presence of antigens that are pathogenic or foreign to the organisms. These antibodies and antigens, *i.e.,* immunoreactants, are capable of binding with one another, thereby creating a highly specific reaction mechanism that can be used *in vitro* to determine the presence or concentration of a particular antigen or antibody in a biological sample.

There are several known immunoassay methods using immunoreactants, wherein at least one of the immunoreactants is labeled with a detectable component so as to be analytically identifiable. For example, the "sandwich" or "two-site" technique may involve the formation of a ternary complex between an antigen and two antibodies. A convenient method of detecting the presence of analytes in a sample is to provide an unlabeled antibody bound to a solid phase support such that the complex can readily be formed between the unlabeled antibody and the analyte present in the sample. After washing, the solid support is then contacted with labeled antibodies that also bind to the

analyte. In this example, the amount of labeled antibody associated with the solid phase is directly proportional to the amount of analyte in the test sample.

In a similar assay format, an antigen may be bound to a solid support, which is then contacted with a test solution that may or may not contain antibodies to the antigen. After washing, the solid support is then contacted with labeled antibodies that bind to any antibodies bound to the analyte. The labeled antibodies can then be detected and their presence directly correlated with the presence of the analyte.

An alternative technique is the "competitive" assay. In one example of a competitive assay, the capture mechanism again may use an antibody attached to an insoluble solid phase, but a labeled analyte competes with the analyte present in the test sample for binding to the immobilized antibody. Similarly, an immobilzed analyte can compete with the analyte of interest for a labeled antibody. In these competitive assays, the quantity of captured labeled reagent is inversely proportional to the amount of analyte present in the sample.

A multi-analyte system should involve a means of providing simultaneous analysis of several analytes in a test sample. This analysis should provide results that identify individual analytes and enable the quantitation of each individual analyte in that test sample. A method of multi-analyte analysis is often claimed but the given criteria are generally not both fulfilled.

In a multi-analyte system, a typical substrate contains a plurality of individual test reaction sites each possessing a different binding ligand. The test sample contacts each of the reaction zones and thereafter a variety of detection techniques can be implemented to identify the analyte(s) present. It is preferable that the detection method used enables quantitation of each individual analyte.

Conventional assay methods often use the values obtained from the reaction of a negative control biological material (e.g. sera) with the multi-analyte panel. Often, multiple negative control sera are used. These negative control sera, obtained from several individuals and different from those providing the sample sera, are used to correct for non-specific reaction of sera in the multi-analyte assay. Problems may arise due to

the fact that the source of the negative samples is different from that of the unknown sample, resulting in unexpected reactivity.

Thus, conventional methods of determining the negative control value may produce an inaccurate estimate of the true negative value. As a result, for some samples, the multi-analyte assay may produce false positive or false negative results.

Brief Summary

The present invention provides unique and advantageous methods for selecting the negative control value to be used when correcting the results obtained from measuring the reaction of a complex biological mixture in a multi-analyte assay.

The methods of the subject invention are particularly advantageous because the negative control value(s) is/are generated using the same sample that is also being analyzed for the presence of the analytes of interest.

In one embodiment, the subject invention pertains to the testing of a blood serum sample using indirect detection methods for immunofluorescence or ELISA type assays. This method provides for a negative control value that, when incorporated into sample calculations, reliably determines whether the unknown sera contains specific antibodies against any of the antigens bound to a solid support.

Detailed Disclosure

The subject invention advantageously provides new and reliable methods for selecting the negative control value(s) to be used in multi-analyte assays. Use of the methods of the subject invention can reduce the incidence of false positives and/or false negatives, particularly in multi-analyte assays conducted on complex biological samples.

Advantageously, in accordance with the practice of the subject invention, the negative control value(s) can be selected from the results obtained only with the sample biological material (e.g. patient sera), without the need for processing negative control material (e.g. normal sera).

In one embodiment, the practice of the subject invention involves utilizing one or more "negative reagents" as the binding ligand in the assay. As used herein, "binding

ligand" or "capture reagent" refers to an entity attached to a solid support and which specifically binds to a target analyte, except for the negative control reagent, which is a reagent for which it is known that the sample of interest does not contain a chemical entity that would specifically bind to the reagent. Thus, this negative control reagent could be, for example, an antigen for which it is known that the sample of interest does not contain an antibody that specifically binds to the antigen. For example, a non-human HLA protein could be used as the negative control reagent for a test wherein the sample of interest is a human sample. Preferably, the negative control reagent is an entity that has physical, chemical, and/or antigenic properties in common with at least one capture reagent. The common properties may be, for example, molecular weight, charge, solubility, tertiary structure and/or conformation. The negative control reagent may be a homolog, ortholog or other such related molecule to the capture reagent.

It is, of course, ideal to find a negative control reagent that generally behaves the same as the reagents used to detect the specific target analyte(s). However, with any biological assay, there is always the possibility of encountering samples having non-specific variable interactions that pass unrecognized and affect the interpretation of the assay. Thus, to reliably interpret the results of any assay, it is advantageous to not only have within one's repertoire the use of negative control reagents, but multiple techniques for determining negative control values (NCVs).

In a further embodiment, the methods of the subject invention can be used to determine an appropriate NCV even without utilizing either traditional negative control sera or the negative control reagent system as described herein. In this embodiment, reactivity values for one or more low-reacting specific target analytes in a multi-analyte assay are used to establish the NCV(s).

In an embodiment specifically exemplified herein, the methods of the subject invention can be applied to the testing of a blood serum sample using indirect detection methods for immunofluorescence or ELISA type assays. This method provides for identification of a NCV that, when incorporated into sample calculations, reliably determines whether an unknown sera contains specific antibodies against any of the

capture reagents bound to a solid support. The solid support may be, for example, beads, wells, membranes, or microarrays.

In one specific embodiment of the subject invention, the panel of binding ligands includes, in addition to the capture reagents for the specific target analytes, one or more non-human proteins or other compounds to neutralize possible charge or chemical interactions that may occur with the solid support. Examples of this type of compound include proteins such as albumin or casein or chemicals that bind or react with the solid support (e.g. if the solid support is a carboxylated plastics it is possible to use chemicals containing amino groups such as ethanolamine or Tris (hydroxymethyl) aminomethane).

All patents, patent applications, provisional applications, and publications referred to or cited herein are incorporated by reference in their entirety, including all figures, to the extent they are not inconsistent with the explicit teachings of this specification.

Following are examples that illustrate procedures for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

Example 1 – Screening for anti-HLA antibodies in human sera

One or more Human Leukocyte Antigens (HLAs) are attached to a solid surface (e.g. multiple wells of a microtiter plate, or different color or size beads) creating a panel of HLAs. This panel of multiple wells or beads also includes negative controls consisting of one or more wells or beads with molecules that are not HLAs. Patient sera is added to all the wells, or mixed with the different beads, and incubated. After washing, the presence of anti-HLA antibodies can be measured by reaction to a labeled anti-immunoglobulin antibody.

Conventional methods produce a negative control by performing several assays using sera from individuals that do not contain anti-HLA antibodies. This is done in order to estimate the amount of non-specific reactivity between sera and capture reagents

(antigens) on the solid support. Typically, the median or average values are used to establish a negative control value (NCV).

U.S. Patent No. 6,046,013 describes a typical process for obtaining the negative control value using a similar type of assay.

It would be extremely rare for an individual to have anti-HLA antibodies against every antigen in the panel; therefore, in one embodiment of the subject invention, the antibody/antigen complex with the lowest signal can be considered the raw NCV. There may be several analytes with similar values; however, since additional calculations will be performed to compensate for assay variability, in one embodiment it is sufficient to use the lowest value. Alternatively, an average of multiple low values can be used. These "low" values would be clearly distinguishable from higher values that reflect a positive result for a particular analyte. The "low" values would, for example, be within 20% of the lowest value and, preferably, within 10% of the lowest value.

The raw NCV may be further adjusted by subtracting the background value (BGV) (i.e. inherent reactivity of the solid support) to obtain a calculated NCV.

The BGV reflects the inherent reactivity of each capture reagent, with the detection antibody. This can be measured by adding water or buffer instead of serum sample to the multi-analyte panel. The BGV may be the same or vary significantly between capture reagents. If there are large differences between the BGVs, each analyte measurement can be corrected by subtracting its own BGV. Alternatively, it is possible to use the same median BGV or average BGV for all analytes.

Another way to determine the NCV for non-specific binding of sample sera to the solid support is by evaluating the reactivity of the sample with one or more non-human HLA-like proteins. In a specific embodiment, leukocyte antigens from non-primates can be attached to a solid support to create non-specific interactions similar to those produced by HLA.

Example 2 — Determination of Negative Control Value

Table 1 shows how to identify the NCV in accordance with one embodiment of the methods of the subject invention.

An "analyte panel" was created by attaching 17 capture reagents to a solid support. A positive (#16) and negative (#17) control were included on the panel.

The sample and water were contacted with the analyte panel (the panel of capture reagents) and subsequently rinsed to wash away unbound molecules. A label was added to detect the attached molecules. The label was detected and the intensity or amount (e.g., absorbance, fluorescence) recorded as shown in Table 1.

The value recorded from each reaction of water and capture reagent is an individual BGV. The average BGV of 26 could be used, but the BGVs had a broad range; therefore, each individual BGV was subtracted from the raw value recorded from each reaction of the sample. These adjusted values were compared to the negative control (#17).

A review of Table 1 reveals that the lowest adjusted value was the reaction of the sample with capture reagent #4 (123) instead of the negative control #17 (530). Therefore, the value for analyte #4 can be used as the negative control instead of the value for analyte #17. Otherwise other analytes would end up as false negatives.

In this example, the raw NCV is the raw value of the sample reacted with capture reagent #4 (158). The calculated NVC is identified by subtracting the raw value of water reacted with capture reagent for analyte #4 (35) from the raw value of the sample reacted with the capture reagent for analyte #4 (158). Therefore, the NCV is 123.

Table 1.

The "analyte panel" contains capture reagents for the detection of 15 different analytes plus one positive and one negative control.

	Analyte panel >	Analyte #1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Water BG:	Individual **BG** Values >	36	11.5	12	35	24	10	89	32	5	6	1	33	13	51	10	17.5	54
Sample:	Values w/each analyte >	291	166	443	**158**	296	300	495	543	595	847	2549	1568	356	461	271	1500	584
					raw NCV													
Sample:	Adjusted Values >	255	154	431	123	272	290	406	511	590	841	2548	1535	343	410	261	1483	530
					calc NCV													
Average BG =	26	(not used in this example)															^ positive control	^ negative control

5

Example 3 —Determination of Negative Control Value

Table 2 shows another example of how to identify the NCV in accordance with the methods of the subject invention.

The same panel for the detection of 17 analytes used in Example 2 was used in this example. This panel included a positive (#16) and a negative (#17) control. However, a different sample was used in this example. In this case, the lowest adjusted value was that obtained from the reaction of the sample with the negative control #17. Therefore, in this case, the adjusted value for #17 would be used as the NCV. Otherwise, many analytes would end up as false negatives.

Table 2.

The "analyte panel" contains capture reagents for the detection of 15 different analytes plus one positive and one negative control.

	Analyte panel >	Analyte #1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Water BG:	Individual **BG** Values >	36	11.5	12	35	24	10	89	32	5	6	1	33	13	51	10	17.5	54
Sample:	Values w/each analyte >	291	166	443	158	296	300	495	543	595	847	2549	1568	456	461	471	1500	**84 raw NC**
Sample:	Adjusted Values >	255	154	431	123	272	290	406	511	590	841	2548	1535	443	410	461	1483	**30 calc NC**
Average BG =	26	(not used in this example)															^ positive control	^ negative control

Example 4

Conventional methods utilize a single "negative reagent" to determine the level of non-specific interaction of a sample in a multi-analyte assay. This is done in order to estimate the amount of non-specific reactivity between sera and the multi-analyte solid support. However, with any biological assay, there is always the possibility of encountering samples having non-specific variable interactions. These interactions may occur with one "negative reagent" but not with a chemically different "negative reagent". Thus, it is advantageous to have within one's repertoire multiple types of "negative reagent" controls (NRC).

In one preferred embodiment, two or more different NRC are included in a multi-analyte panel. After the assay with the sample sera, a calculated NC value is obtained for each NRC. The calculated NC value for each NRC is used separately to evaluate the reactivity of the sample against the analytes in the panel. This produces as many sets of results as the number of NRCs included in the multi-analyte panel. The comparison of these sets shows which analytes are consistently positive or negative with the different NRCs.

For example, if four independent NRCs are included in the multi-analyte panel, four sets of results are generated. By counting the number of times that an analyte generates the same type of results in the four sets, it is possible to determine the concordance or consistency of the results.

One method to interpret the results is to count the number of times an analyte is positive in the four sets. If the analyte is positive in 3 or 4 sets of results, it would be considered Positive. If it was positive in 2 sets it would be considered as Tentative or Doubtful Positive. If it was positive in 1 set it would be considered as Tentative or Doubtful Negative. If it was not positive in any of the sets in would be considered Negative.

Example 5 – Effect of Multiple Control beads on Results from an Antibody Screening Assay

A serum sample was assayed using the Lifematch Antibody Screen assay (Tepnel Lifecodes, Stamford, CT), designed for use with a Luminex 100 fluoranalyzer, which was modified to include three negative control beads. This product consists of one bead population with HLA Class I antigens attached, a second population with Class II antigens attached, a third population with Human IgG that serves as a positive control for the detection reagent, a fourth population that contains an analyte not related to HLA that serves as negative control (CON1), a fifth population that contains a different analyte as a second negative control (CON2) and a sixth population that contains yet a different analyte that serves as a third negative control (CON3).

As described below, the median fluorescent intensity (MFI) for each antigen-containing bead (i.e., Class I or Class II beads) is divided by the MFI of each negative control bead yielding 3 quotients. From each quotient is subtracted a background adjustment factor (BAF) that is provided by the kit manufacturer. This results, for each analyte, in three *adjusted values* (AdjVal). If any adjusted value is greater than zero, a positive reactivity for an antigen-containing bead is indicated. Table 3 illustrates the results obtained for this example assay.

Table 3

Bead	MFI	BAF	AdjVal1 (a)	AdjVal2 (b)	AdjVal3 (c)	Result
Class I	10,137.5		52.92	29.94	28.97	Positive
CON1	182	2.785				
CON2	311.5	2.608				
CON3	323.5	2.367				
Class II	652		0.51	-0.92	-0.76	Positive
CON1	182	3.068				
CON2	311.5	3.009				
CON3	323.5	2.774				

(a) AdjVal1 = (MFI/CON1) – $BAF_{CON\ 1}$;
(b) AdjVal2 = (MFI/CON2) – $BAF_{CON\ 2}$;
(c) AdjVal3 = (MFI/CON3) – $BAF_{CON\ 3}$

For Class I, a positive reaction is indicated by all three adjusted values, whereas for Class II, a positive reaction is indicated for only one adjusted value. Because two of the negative control beads (CON2 & CON3) show a higher background than CON1, they yield a false negative result.

As shown in this example, if only CON2 or CON3 were present in the assay, this sample would have been incorrectly assigned as having no antibody toward Class II antigens. Thus, inclusion of multiple negative control beads reduces the likelihood of a false negative assignment.

Example 6 — Application of the lowest signal analyte as Negative Control to compensate for effect of high background

This example illustrates the effect of employing the lowest signal analyte as negative control for the analysis of a sample that has a high background with the Negative Control Bead.

A serum sample containing antibodies toward HLA-A2 and HLA-A68 antigens was assayed using the Lifematch Class I ID kit (Tepnel Lifecodes, Stamford, CT) and analyzed with a Luminex 100 fluoroanalyzer. The assay was performed according to the product insert. Briefly, the product contains a Negative Control Bead, a Positive Control Bead and a series of beads that contain various combinations of antigens. The assay comprises 1) mixing a serum sample with the beads, 2) washing away unbound sera, 3) adding a label that binds to the captured HLA-antibodies and 4) detecting the amount of label bound to each bead with the Luminex 100. The amount of label bound is reported as median fluorescence intensities (MFI).

For this example, the MFI values for each analyte is shown in Table 4. The results show that this sample has a high non-specific interaction with the Negative Control (CON1) bead.

In one method of analysis, the MFI for each antigen-containing bead is divided by the MFI of CON1. From this quotient is subtracted a background adjustment factor

(BAF) that is provided by the kit manufacturer. A resultant greater than zero indicates a positive reactivity for an antigen-containing bead.

The use of this calculation for CON1 results in the classification of only 2 analyte containing beads as positive. As such, the sample may be erroneously classified as antibody negative. In addition, the concordance between the positive beads and a particular analyte (e.g. HLA-A antigen) in the bead is insufficient for antibody identification.

An alternative method for calculating a positive reaction employs an antigen-containing bead as a negative control. For most assays, a serum sample will not have a positive reaction with all antigen-containing beads. Thus, the antigen-containing bead that has the lowest MFI reading can be employed as a negative control to calculate an adjusted value (AdjVal2).

To determine reactivity, the same calculation is applied to the antigen-containing beads except that the MFI of CON1 bead is replaced by the MFI of the lowest value bead. In this same example, using the MFI of the lowest value bead (#156), results in the classification of 26 analyte containing beads, as positive (AdjVal2; Table 4). An examination of the analytes present in those beads shows 100% correlation with the presence of HLA-A2 and HLA-A68 on the beads. Therefore, in samples with high values for CON1, the analysis of the results using the lowest value analyte containing bead, allows for the correct identification of the antibodies present in the sample.

Table 4

Bead ID	MFI Sample B	AdjVal1	AdjVal2	BAF	Antigens Present on Bead							
121	16867	**1.30**	**4.09**	1.951	A2	--	B39	B70	-	Bw6	Cw7	Cw12
165	10919	**0.05**	**1.86**	2.054	A68	A30	B18	B35	-	Bw6	Cw4	Cw5
177	10860	**-0.00**	**1.80**	2.092	A2	A3	B35	B35	-	Bw6	Cw3	Cw15
119	10516	**-0.01**	**1.73**	2.039	A2	A3	B50	B57	Bw4	Bw6	Cw6	Cw18
134	10711	**-0.06**	**1.71**	2.123	A2	--	B27	B56	Bw4	Bw6	Cw1	--
130	10127	**-0.03**	**1.65**	1.978	A24	A68	B18	B27	Bw4	Bw6	Cw1	Cw7
151	9561.5	**-0.04**	**1.55**	1.878	A2	A25	B8	B27	Bw4	Bw6	Cw1	Cw7
139	9567	**-0.18**	**1.40**	2.023	A2	A29	B13	B62	Bw4	Bw6	Cw3	Cw6
135	9608	**-0.25**	**1.35**	2.096	A2	A30	B13	B44	Bw4	-	Cw5	Cw6
122	9143.5	**-0.23**	**1.29**	1.99	A24	A68	B60	B65	-	Bw6	Cw3	Cw8
118	9078	**-0.23**	**1.27**	1.979	A2	A24	B8	B50	-	Bw6	Cw6	Cw7
149	9015	**-0.43**	**1.06**	2.17	A2	A74	B53	B70	Bw4	Bw6	Cw2	Cw6
168	9034.5	**-0.47**	**1.02**	2.215	A1	A2	B45	B70	-	Bw6	Cw3	Cw16
102	7973	**-0.45**	**0.87**	1.982	A2	A34	B8	B65	-	Bw6	Cw2	Cw7
126	8006	**-0.46**	**0.87**	1.998	A2	A23	B39	B62	-	Bw6	Cw3	Cw7
108	7063	**-0.55**	**0.62**	1.907	A2	A33	B42	B63	Bw4	Bw6	Cw14	Cw17
167	7648.5	**-0.71**	**0.56**	2.182	A2	A68	B18	B52	Bw4	Bw6	Cw2	Cw16
147	6624.5	**-0.63**	**0.47**	1.903	A2	A33	B44	B51	Bw4	-	Cw5	Cw15
109	7145	**-0.72**	**0.46**	2.096	A2	A11	B8	B60	-	Bw6	Cw3	Cw7
117	8042.5	**-0.89**	**0.44**	2.437	A3	A68	B7	B35	-	Bw6	Cw4	Cw7
159	6671	**-0.70**	**0.41**	1.985	A2	A24	B7	B61	-	Bw6	Cw2	Cw12
128	6140	**-0.68**	**0.34**	1.859	A2	A24	B48	B62	-	Bw6	Cw3	Cw8
111	6541.5	**-0.75**	**0.34**	2.007	A68	A33	B7	B40	-	Bw6	Cw7	Cw15
164	6116.5	**-0.72**	**0.29**	1.897	A2	A32	B39	B60	-	Bw6	Cw3	Cw12
124	5854.5	**-0.68**	**0.29**	1.805	A2	A30	B51	B62	Bw4	Bw6	Cw3	Cw15
154	5048	**-0.81**	**0.03**	1.779	A11	A29	B49	B58	Bw4	-	Cw4	Cw7
104	4827	**-0.92**	**-0.12**	1.845	A1	A26	B55	B57	Bw4	Bw6	Cw3	Cw6
112	4882	**-1.06**	**-0.25**	1.997	A30	A33	B52	B58	Bw4	-	Cw4	Cw16
105	4956	**-1.09**	**-0.26**	2.04	A11	A29	B35	B46	-	Bw6	Cw1	Cw4
153	4868.5	**-1.10**	**-0.29**	2.033	A24	A33	B44	B62	Bw4	Bw6	Cw3	Cw16
137	4554	**-1.04**	**-0.29**	1.921	A30	A33	B42	B82	-	Bw6	Cw4	Cw7
148	4700.5	**-1.07**	**-0.29**	1.977	A11	A32	B44	B55	Bw4	Bw6	Cw3	Cw5
106	5081	**-1.16**	**-0.32**	2.14	A25	A30	B13	B18	Bw4	Bw6	Cw6	Cw12
142	4781	**-1.11**	**-0.32**	2.033	A26	A30	B51	B62	Bw4	Bw6	Cw3	Cw14
107	5297	**-1.20**	**-0.32**	2.222	A1	A66	B41	B65	-	Bw6	Cw8	Cw17
144	4125	**-1.03**	**-0.35**	1.828	A24	A32	B27	B61	Bw4	Bw6	Cw1	Cw2
138	4266.5	**-1.10**	**-0.39**	1.922	A1	A31	B8	B35	-	Bw6	Cw4	Cw7
157	4066.5	**-1.08**	**-0.41**	1.863	A1	A11	B44	B70	Bw4	Bw6	Cw5	Cw7
103	4351	**-1.14**	**-0.42**	1.982	A1	A11	B8	B62	-	Bw6	Cw7	Cw8
163	4397.5	**-1.15**	**-0.43**	2.001	A23	A33	B7	B18	-	Bw6	Cw5	Cw15
150	4374	**-1.16**	**-0.43**	2.002	A3	A32	B18	B37	Bw4	Bw6	Cw5	Cw6
175	4343	**-1.21**	**-0.49**	2.051	A26	A33	B8	B78	-	Bw6	Cw3	Cw16

Table 4

Bead ID	MFI Sample B	AdjVal1	AdjVal2	BAF	Antigens Present on Bead							
161	4648	**-1.27**	**-0.50**	2.162	A24	A32	B35	B63	Bw4	Bw6	Cw4	Cw7
160	4657	**-1.29**	**-0.52**	2.185	A23	A31	B41	B44	Bw4	Bw6	Cw4	Cw15
132	4387.5	**-1.25**	**-0.52**	2.096	A32	A74	B64	B65	-	Bw6	Cw8	--
129	3487.5	**-1.22**	**-0.64**	1.893	A29	A34	B44	B61	Bw4	Bw6	Cw4	Cw8
152	4663	**-1.42**	**-0.65**	2.316	A23	A80	B45	B81	-	Bw6	Cw16	Cw18
169	3637	**-1.26**	**-0.66**	1.963	A23	A36	B53	B58	Bw4	-	Cw4	Cw7
173	4708	**-1.44**	**-0.66**	2.35	A1	A24	B38	B70	Bw4	Bw6	Cw7	Cw12
156	2791	**-1.55**	**-1.09**	2.091	A3	A24	B7	B60	-	Bw6	Cw3	Cw7
CON1	5192											

It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application.